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Growth and Persistence of Pathogens on Granular Activated Carbon Filters

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Three enteric pathogens *Yersinia enterocolitica* O:8, *Salmonella typhimurium*, and enterotoxigenic *Escherichia coli*, were examined for their ability to colonize granular activated carbon (GAC) in pure cultures and in the presence of autochthonous river water organisms. All three organisms readily colonized sterile GAC and maintained populations of ca. 10^5 to 10^7 CFU g^{-1} for 14 days when suspended in sterile river water. Exposure of pathogen biofilms on GAC to unsterile river water resulted in a gradual decline in pathogens on the carbon (0.08 to 0.14 log day^{-1}). When pathogens were introduced to sterile GAC in the presence of heterotrophic plate count organisms, they attached at levels similar to those in the pure cultures and then decreased (0.10 to 0.22 log day^{-1}). When added with heterotrophic plate count bacteria to GAC supporting a mature biofilm of native river water bacteria, they attached at a lower level (1.0×10^4 to 4.6×10^4 CFU g^{-1}) and decreased at a more rapid rate (0.11 to 0.70 log day^{-1}).

Carbon has been used as an adsorbent for centuries. In 1930, meat packing industries in Chicago began to use activated carbon to correct disagreeable tastes and odors in water effluents (11). By 1938, over a thousand water treatment plants in the U.S. were using carbon as part of their process (11). In 1970, the Community Water Supply Survey (16) revealed that unpleasant tastes and odors in drinking water were the most common customer complaint. Consequently, powdered activated carbon and granular activated carbon (GAC) have been widely used to combat taste, odor, and color problems in surface water caused by wastes from industrial, municipal, and agricultural sources, decaying vegetation, and algal blooms (11, 19, 22). As a result of amendments to the *National Interim Primary Drinking Water Regulations* (9, 10), many water treatment systems used powdered activated carbon or GAC to control organic residues such as oil, gasoline, phenol, or trihalomethanes and their precursors (18, 19).

The high degree of transition porosity in activated carbon allows for an increase in the diffusion-limited rate of adsorption from solutions and makes it very effective in adsorbing a wide range of organic compounds which provide nutrients for bacterial attachment and growth on these particles (2, 4, 5, 7, 15, 24, 26). Carbon particles may be densely colonized (2, 4, 7, 21, 24, 26), and problems may arise when particles penetrate treatment barriers and enter finished drinking water.

We have recently reported methods to desorb viable bacteria from GAC (6) and have used this technique to demonstrate that there is greatly reduced disinfection efficiency with chlorine when bacteria are attached to GAC (13). We have also identified *Salmonella* sp. on a GAC filter and *Escherichia coli* producing a borderline enterotoxic reaction with suckling mice from carbon particles in GAC-treated drinking water (13; A. K. Camper, D. G. Davies, S. C. Broadaway, M. W. LeChevallier, and G. A. McFeters, Abstr. Annu. Meet. Am. Soc. Microbiol. 1985, N38, p. 223). In addition, 30% of the 265 coliform isolates from carbon particles in drinking water exhibit the fecal biotype (Camper et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1985).

The present study was initiated to address questions concerning the growth and persistence of various waterborne pathogens (*Yersinia enterocolitica*, *Salmonella typhimurium*, and enterotoxigenic *E. coli*) on GAC filters. It was found that the autochthonous microbial community profoundly influenced the survival of the pathogens, but that the pathogens could survive for extended periods of time when virgin carbon was used. These results will have an impact on how activated carbon is used to treat drinking water.

MATERIALS AND METHODS

Bacterial strains. The *Y. enterocolitica* strain (O:8) used in these studies was obtained from Donald A. Schiemann, Montana State University. *S. typhimurium* isolated from a disease outbreak was received from the Wisconsin State Laboratory of Hygiene, Madison. A human enterotoxigenic *E. coli* strain (0142) was provided by the *E. coli* Reference Center, Pennsylvania State University, University Park. This organism was resistant to erythromycin, lincomycin, penicillin, and tetracycline. All cultures were stored at −70°C in a 20% glycerol–2% peptone solution. Cells were grown in TLY broth (17) for 24 h. *S. typhimurium* and *E. coli* were incubated at 35°C, and *Y. enterocolitica* was grown at room temperature (ca. 20 to 22°C). Cells were harvested by centrifugation at 3,020 × *g* for 10 min, and the pellet was resuspended in cold, sterile Milli-Q reagent grade water (Millipore Corp., Bedford, Mass.).

Experimental apparatus. All columns were initially prepared by packing sterile Plexiglas tubes (15 by 2.5 cm; Rohm & Haas Co., Philadelphia, Pa.) with 12.5-cm GAC (Nuchar, 8/30 mesh; Westvaco Chemical Division, Covington, Va.) that had been autoclaved at 121°C for 20 min. All columns were covered with foil and maintained in the dark at room temperature (ca. 20 to 22°C).

River water was obtained from the East Gallatin River near Bozeman. This was used either after autoclaving at 121°C for 15 min or as collected.

Water was continuously circulated through the GAC columns by means of a peristaltic pump (Harvard Apparatus

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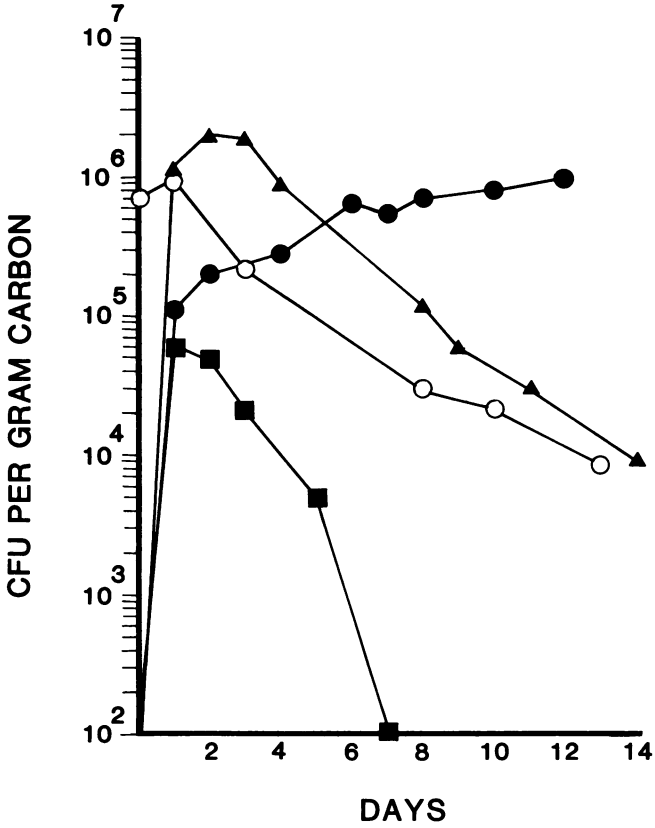

FIG. 1. Attachment and persistence of *Y. enterocolitica* O:8 on GAC columns. Symbols: ●, sterile river water, sterile carbon; ○, continuation of above, nonsterile river water added; ▲, nonsterile river water, sterile carbon; ■, nonsterile river water, precolonized carbon.

Co., Inc., Millis, Mass.) at a flow rate of 27 ml min^{-1}. A 2-liter catch flask was used as the water reservoir.

Sampling techniques. GAC cores were removed from the columns with sterile glass tubing that was long enough to sample the entire depth of the column. The carbon was rinsed three times with sterile, reagent grade water to remove unattached organisms and drained on sterile filter paper, and 1 g was removed and weighed. The carbon was suspended in 100 ml of cold sterile reagent grade water, and the attached bacteria were desorbed as described previously (6). This technique involves the homogenization of the carbon particles at 16,000 rpm for 3 min at 4°C with a mixture of Zwittergent 3-12 (10^{-6} M) (Calbiochem-Behring, La Jolla, Calif.), ethylene glycol-bis-(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA) (10^{-3} M), Tris buffer (0.01 M, pH 7.0), and peptone (0.1%). The homogenization procedure removes cells attached to the GAC and prevents their readsorption. Circulated water samples were also assayed for planktonic bacteria.

All samples were enumerated by the spread plate technique in triplicate (1). Plate count agar (Difco Laboratories, Detroit, Mich.) was used to determine the heterotrophic plate count (HPC) bacterial population. Incubation was at 35°C for 48 h. *Y. enterocolitica* was enumerated on yersinia selective agar base plus supplement (Oxoid Ltd., London, England). *S. typhimurium* was plated on brilliant green agar (Difco). *E. coli* with resistance markers for tetracycline and lincomycin was grown on mT7 agar (12) containing 8 mg of tetracycline $liter^{-1}$ and 1 mg of lincomycin $liter^{-1}$ (Sigma Chemical Co., St. Louis, Mo.). All media were incubated for 24 h at 35°C. The selective media were chosen because they allowed high recoveries of target organisms but prevented growth of the indigenous river organisms.

Experimental design. Three experimental designs were devised to determine the attachment, growth, and persistence of the enteric pathogens on GAC columns in the presence and absence of HPC organisms. One system was designed to examine the interaction of the test organism with sterile GAC. An inoculum (ca. 10^4 cells ml^{-1}) was added to the circulating sterile river water. Samples of eluted water and GAC were removed, and the pathogens were enumerated at timed intervals. After at least 2 weeks, the column was rinsed with sterile water, and the circulated water was replaced with nonsterile river water. In this way the survival of a pure culture of attached pathogens in the presence of introduced HPC organisms could be assessed. Sampling continued until a noticeable decline in pathogen cell counts was observed. Another procedure examined the ability of pathogens to compete with HPC organisms when simultaneously introduced to sterile GAC. Nonsterile river water with ca. 10^4 added pathogens ml^{-1} was circulated through a column. Samples were removed and assayed at regular intervals. The last procedure used a GAC column which had been precolonzied with HPC organisms. The mature biofilm was established by circulating river water through the column for at least 2 weeks. A stable HPC population of ca. 10^8 cells g of GAC^{-1} was obtained. The system was rinsed with sterile reagent grade water, and fresh river water containing ca. 10^4 pathogens ml^{-1} was pumped through the column. Bacteria on the GAC and in the water were enumerated on a regular basis until pathogen numbers fell below detection limits.

RESULTS

Three waterborne enteric pathogens were selected for use in this study. The ability of these organisms to attach and persist on GAC drinking water filters with and without the presence of indigenous surface water bacteria was examined by using three experimental designs.

Y. enterocolitica. When added to the sterile system (virgin GAC, sterile surface water), this pathogen attained a level of 10^5 cells g of GAC^{-1} within 1 day, and the number of attached cells increased by 1 log during the next 11 days (Fig. 1). A near constant number of cells was recovered in the effluent (ca. 5×10^3 cells ml^{-1}). The introduction at day 14 of heterotrophic river water organisms resulted in a gradual decline (0.17 log day^{-1}) of the pathogens on the GAC. By day 13 pathogen levels had decreased to 10^4 cells g of GAC^{-1}. The number of *Y. enterocolitica* cells shed into the aqueous phase declined in a similar manner. In contrast, the HPC organisms increased in number on the GAC during the first 2 days and established a stable population of nearly 10^8 cells g of GAC^{-1}. Recovery of HPC bacteria from the water column was constant at ca. 10^5 cells ml^{-1}. Similar results for HPC concentrations were observed in all of the following experiments.

The next protocol examined the capability of *Y. enterocolitica* to compete with an established population of normal river water flora for attachment sites on sterile GAC. The pathogen reached a level of 10^6 CFU g of GAC^{-1} after 24 h. The cell numbers peaked at day 2 and then decreased at a rate of 0.18 log day^{-1} over the next 12 days (Fig. 1). The

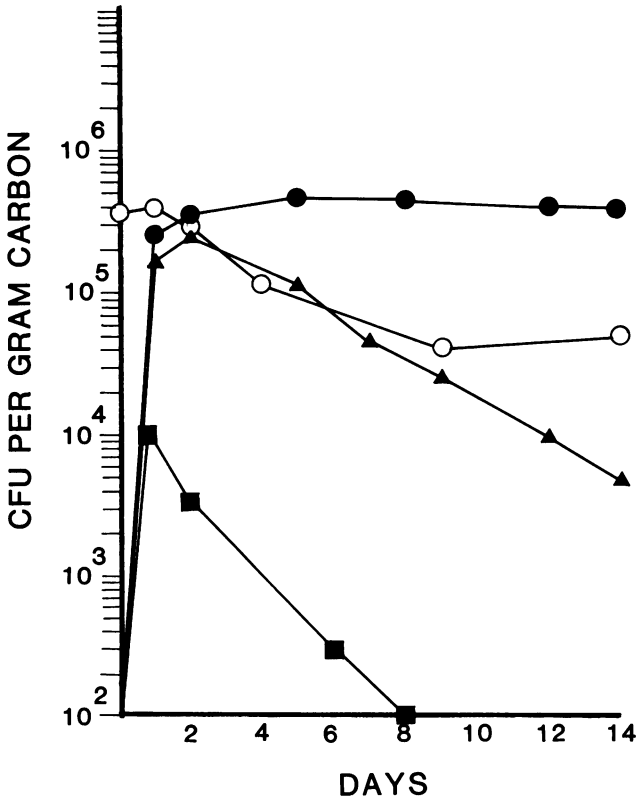

FIG. 2. Attachment and persistence of *S. typhimurium* on GAC columns. Symbols: ●, sterile river water, sterile carbon; ○, continuation of above, nonsterile river water added; ▲, nonsterile river water, sterile carbon; ■, nonsterile river water, precolonized carbon.

level of planktonic pathogens decreased at a rate of 0.52 log day^{-1}.

The presence of a mature biofilm of autochthonous organisms from surface water on GAC prevented *Y. enterocolitica* from establishing at concentrations found in the previous experiments (Fig. 1). The initial attached population (5×10^4 cells g of GAC^{-1} at 24 h) declined by 2.5 log in 7 days. *Y. enterocolitica* suspended in the water column also declined by 2.5 log in 7 days.

S. typhimurium. The capacity of *S. typhimurium* to colonize GAC was examined by using the same experimental design (Fig. 2). *S. typhimurium* cells suspended in sterile river water pumped through sterile GAC quickly attached and attained a density of 5×10^5 bacteria g of GAC^{-1}. This population was maintained at a constant level for the 2-week sampling time. Planktonic cell counts were also constant at ca. 3×10^3 cells ml^{-1}. *S. typhimurium* attached to GAC were slowly eliminated after the addition of river water containing autochthonous bacteria. The magnitude of this decrease was 1 log of pathogens g GAC^{-1} from days 2 to 9. Circulated water contained a constant 6×10^2 pathogens ml^{-1} for the entire sampling time.

When a mixed population of *S. typhimurium* and HPC organisms was circulated through sterile GAC, the pathogen attached to the carbon, and by day 2 it had established a density of 1.5×10^5 cells g^{-1} (Fig. 2). Over the next 2 weeks the *S. typhimurium* population was reduced by 0.1 log day^{-1}. Cell counts in the eluted water declined at a rate of 0.4 log day^{-1} during week 1 and then at 0.06 log day^{-1} for the remainder of the experiment. These data indicated that the suspended pathogen cells died at a faster rate than did those attached to GAC.

When *S. typhimurium* was added to nonsterile river water circulating through GAC with a mature HPC biofilm, the pathogen attached at a level of ca. 10^4 CFU g of GAC^{-1} (Fig. 2). *S. typhimurium* cells were eliminated from the GAC at a rate of 0.25 log day^{-1}. A biphasic curve with rates similar to those of the previous situation was observed for cells suspended in the water column.

Enterotoxigenic *E. coli*. Enterotoxigenic *E. coli* in a sterile system attached at a level of ca. 10^7 cells g of GAC^{-1} and maintained this level for 2 weeks (Fig. 3). Circulating *E. coli* existed at a constant number of ca. 1.5×10^5 cells ml^{-1}. Introduction of nonsterile river water resulted in a gradual decline (0.1 log day^{-1}) of pathogens (Fig. 3). On day 20, 3.3×10^5 cells g of GAC^{-1} were detected. Cells suspended in the water column were eliminated more rapidly (at a rate of 0.16 log day^{-1}).

Enterotoxigenic *E. coli* introduced with HPC organisms to sterile GAC established at 1.3×10^6 cells g^{-1}, which was nearly 1 log lower than the value observed for enterotoxigenic *E. coli* alone. In this instance, the pathogen was removed from the GAC more quickly during the first 5 days (ca. 0.26 log day^{-1}) and at a reduced rate until day 20 (0.06 log day^{-1}). Planktonic cells decreased by 0.30 log day^{-1} to day 10 and decreased 1 log further by day 18.

Enterotoxigenic *E. coli* appeared to persist on GAC precolonized with HPC organisms to a greater degree than

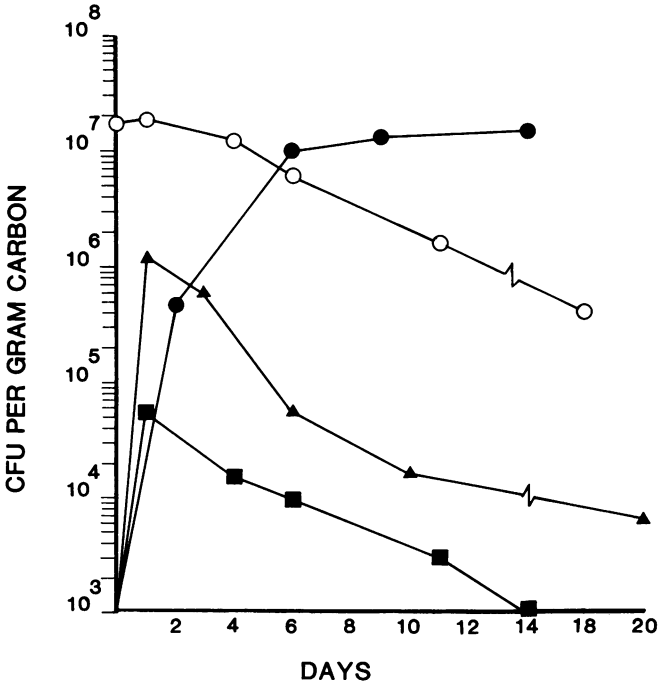

FIG. 3. Attachment and persistence of enterotoxigenic *E. coli* on GAC columns. Symbols: ●, sterile river water, sterile carbon; ○, continuation of above, nonsterile river water added; ▲, nonsterile river water, sterile carbon; ■, nonsterile river water, precolonized carbon.

did the other two pathogens. The initial attachment was similar (4.5×10^4 cells g^{-1}), but the enterotoxigenic *E. coli* persisted considerably longer. The rate of decline was 0.1 log day^{-1}. Suspended bacteria were initially present at a population of 1.1×10^5 cells ml^{-1}. They were eliminated at a rate of 0.41 log day^{-1}.

DISCUSSION

The results of this report have indicated that various waterborne pathogens can survive for extended periods of time when they colonize virgin GAC. The extent of colonization by pathogens is limited by the autochthonous microbial community. In the absence of an autochthonous community, the pathogens colonized and grew on the GAC to levels of 5×10^5 to 1×10^7 CFU g^{-1}. When native aquatic bacteria were added to pathogens growing on sterile GAC, the pathogens declined at rates varying from 0.08 to 0.14 log day^{-1}. When native aquatic bacteria and pathogens were added simultaneously to fresh GAC, the pathogens declined slightly faster (0.10 to 0.22 log day^{-1}). Finally, when pathogens were added to precolonized GAC, they attached but decreased at the fastest rates (0.11 to 0.70 log day^{-1}). These results demonstrate the importance of indigenous organisms in controlling human pathogenic bacteria on GAC particles. Mechanisms of pathogen inhibition on surfaces have been reviewed by Savage (23). Competition for nutrients, space, and production of inhibitory secondary metabolites are some of the mechanisms by which bacteria compete in natural environments. It is known that many bacteria can produce bacteriocins and other factors that influence competition (3, 8, 20, 25). However, competition for nutrients and space was probably the basis for the declines in pathogen levels reported here. These data are supported by a recent report that showed that HPC bacteria could inhibit members of the *Enterobacteriaceae* by competition for available sources of carbon and energy (14).

The data presented here have important implications regarding the use of GAC in the treatment of drinking water. Operators of drinking water facilities should pay particular attention to GAC filters when they are first replaced, since it is during this time that pathogens can easily colonize and persist in the carbon beds. Pretreatment (chlorination, chlorine dioxide, and ozone) is very important during this time to reduce the number of pathogens reaching the GAC filters and should continue until the GAC has developed an indigenous population of 10^6 to 10^8 HPC bacteria g^{-1}. Alternatively, the filter could be left out of operation for 1 or 2 weeks to allow the establishment of an autochthonous microbial biofilm community. We recently collected samples from a treatment plant which used GAC filters 1 week after the carbon had been replaced. The average coliform count in filter effluents (before final chlorination) before the carbon was replaced was 3 cells 100 ml^{-1}, whereas coliform levels rose to >1,300 cells 100 ml^{-1} in the filter effluents after virgin carbon was added (A. K. Camper, M. W. LeChevallier, S. C. Broadaway, and G. A. McFeters, manuscript in preparation).

The results also indicated that human pathogenic bacteria may colonize mature GAC filters, although they decline at a faster rate than on uncolonized carbon. We have previously isolated *S. typhimurium* and enterotoxigenic *E. coli* producing a low level of heat-stable toxin from mature GAC filters (13; Camper et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1985). Although the levels of these pathogens were low, the data indicate the importance of pretreatment, even for filters having a well-developed biofilm to reduce the levels of pathogenic bacteria.

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